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taq adj polymerase.ti.	0

Database: US Patents Full-Text Database; US Pre-Grant Publication Full-Text Database; JPO Abstracts Database; EPO Abstracts Database; Derwent World Patents Index; IBM Technical Disclosure Bulletins

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Today's Date: 5/30/2001

DB Name	Query	Hit Count	Set Name
USPT,PGPB,JPAB,EPAB,DWPI	taq adj polymerase.ti.	0	L13
USPT,PGPB,JPAB,EPAB,DWPI	taq polymerase.ti.	0	L12
USPT,PGPB,JPAB,EPAB,DWPI	L7 and (Phe or Tyr)	94	L11
USPT,PGPB,JPAB,EPAB,DWPI	L7 same Phe	0	L10
USPT,PGPB,JPAB,EPAB,DWPI	L7 same (Phe-667)	0	L9
USPT,PGPB,JPAB,EPAB,DWPI	L7 same (Phe-667) or (Tyr-667)	0	L8
USPT,PGPB,JPAB,EPAB,DWPI	(Taq polymerase)same (mutant or variant)	193	L7
USPT,PGPB,JPAB,EPAB,DWPI	l4 and (position 667)	0	L6
USPT,PGPB,JPAB,EPAB,DWPI	l3 and (Phe or Tyr)	1506	L5
USPT,PGPB,JPAB,EPAB,DWPI	l3 and (Phe-667 or Phe 667)	0	L4
USPT,PGPB,JPAB,EPAB,DWPI	l1 and (mutant or variant)	3029	L3
USPT,PGPB,JPAB,EPAB,DWPI	l1 and (mutant or variant or modifi$)	4055	L2
USPT,PGPB,JPAB,EPAB,DWPI	Taq adj polymerase	4211	L1

Gene 1992 Mar 1;112(1):29-35

The fidelity of Taq polymerase catalyzing PCR is improved by an N-terminal deletion.

Barnes WM

Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110.

KlenTaq DNA polymerase is an N-terminally truncated Thermus aquaticus (Taq) DNA polymerase I. As expressed from a gene construct in Escherichia coli, translation initiates at Met236, bypassing the 5'----3' exonuclease domain of the DNA polymerase-encoding gene. A sensitive forward mutation assay was used to measure the relative number of mutations introduced into the entire lacZ gene by the polymerase chain reaction (PCR) under various conditions which allow the amplification of such a large DNA span. Two selectable markers, one at each end of the test lacZ fragment, were employed to avoid the plating and scoring of PCR artefacts such as primer initiation in the midst of the lacZ gene, and cloning artefacts such as empty vector plasmid. The measured relative mutation rate was twofold lower for KlenTaq as compared to the full-length Taq DNA polymerase.

PMID: 1551596, UI: 92201698

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FEBS Lett 1999 Apr 1;448(1):145-8

Mutation S543N in the thumb subdomain of the Taq DNA polymerase large fragment suppresses pausing associated with the template structure.

Ignatov KB, Bashirova AA, Miroshnikov AI, Kramarov VM

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow. vkram@glasnet.ru

Substitution of Asn for the conserved Ser543 in the thumb subdomain of the Taq DNA polymerase large fragment (Klentaq DNA polymerase) prevents pausing during DNA synthesis and allows the enzyme to circumvent template regions with a complex structure. The mutant enzyme (KlentaqN DNA polymerase) provides specific PCR amplification and sequencing of difficult templates, e.g. those with a high GC% content or strong secondary structure.

PMID: 10217428, UI: 99231780

Biochemistry 1999 Feb 2;38(5):1426-34

Functional roles of the conserved aromatic amino acid residues at position 108 (motif IV) and position 196 (motif VIII) in base flipping and catalysis by the N6-adenine DNA methyltransferase from Thermus aquaticus.

Pues H, Bleimling N, Holz B, Wolcke J, Weinhold E

Max-Planck-Institut fur molekulare Physiologie, Abteilung Physikalische Biochemie, Dortmund, Germany.

The DNA methyltransferase (Mtase) from Thermus aquaticus (M.TaqI) catalyzes the transfer of the activated methyl group of S-adenosyl-L-methionine to the N6 position of adenine within the double-stranded DNA sequence 5'-TCGA-3'. To achieve catalysis M.TaqI flips the target adenine out of the DNA helix. On the basis of the three-dimensional structure of M.TaqI in complex with the cofactor and its structural homology to the C5-cytosine DNA Mtase from Haemophilus haemolyticus, Tyr 108 and Phe 196 were suggested to interact with the extrahelical adenine. The functional roles of these two aromatic amino acid residues in M.TaqI were investigated by mutational analysis. The obtained mutant Mtases were analyzed in an improved kinetic assay, and their ability to flip the target base was studied in a fluorescence-based assay using a duplex oligodeoxynucleotide containing the fluorescent base analogue 2-aminopurine at the target position. While the mutant Mtases containing the aromatic amino acid Trp at position 108 or 196 (Y108W and F196W) showed almost wild-type catalytic activity, the mutant Mtases with the nonaromatic amino acid Ala (Y108A and F196A) had a strongly reduced catalytic constant. Y108A was still able to flip the target base, whereas F196A was strongly impaired in base flipping. These results indicate that Phe 196 is important for stabilizing the extrahelical target adenine and suggest that Tyr 108 is involved in placing the extrahelical target base in an optimal position for methyl group transfer. Since both aromatic amino acids belong to the conserved motifs IV and XIII found in N6-adenine and N4-cytosine DNA Mtases as well as in N6-adenine RNA Mtases, a similar function of aromatic amino acid residues within these motifs is expected for the different Mtases.

PMID: 9931007, UI: 99129939

FEBS Lett 1998 Mar 27;425(2):249-50

Substitution of Asn for Ser543 in the large fragment of Taq DNA polymerase increases the efficiency of synthesis of long DNA molecules.

Ignatov KB, Miroshnikov AI, Kramarov VM

Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow.

Substitution of Asn for Ser543 in the large fragment of Taq DNA polymerase (Klentaq) increases several times the efficiency of synthesis of long (over 2 kbp) DNA molecules. The difference in the DNA synthesis efficiencies by the mutant and native enzymes increased with the increase in the DNA fragment length.

PMID: 9559658, UI: 98218560

=> d his

(FILE 'HOME' ENTERED AT 14:26:27 ON 30 MAY 2001)

INDEX 'ADISALERTS, ADISINSIGHT, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, DRUGNL, ...' ENTERED AT 14:26:37 ON 30 MAY 2001

SEA TAQ(W)POLYMERASE

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L1 QUE TAQ(W) POLYMERASE

FILE 'MEDLINE, CAPLUS, BIOSIS, TOXLIT' ENTERED AT 14:28:14 ON 30 MAY 2001

L2 518 S L1 A[redacted](MUTANT OR VARIANT OR MODIFI?)[redacted]
L3 0 S L2 AND (TYR667 OR PHE667)
L4 0 S L2 AND (POSITION(W)667)
L5 5 S L2 AND (PHE OR TYR)
L6 3 DUP REM L5 (2 DUPLICATES REMOVED)
L7 0 S L2 AND (PHE-667 OR PHE(W)667)
L8 0 S L2 AND (PHE-570 OR PHE(W)570)

=> d 16 ibib ab 1-3

L6 ANSWER 1 OF 3 MEDLINE DUPLICATE 1
ACCESSION NUMBER: 1999380545 MEDLINE
DOCUMENT NUMBER: 99380545 PubMed ID: 10449720
TITLE: Structure-based design of Taq DNA polymerases with improved properties of dideoxynucleotide incorporation.
AUTHOR: Li Y; Mitaxov V; Waksman G
CORPORATE SOURCE: Department of Biochemistry and Molecular Biophysics, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis, MO 63130, USA.
CONTRACT NUMBER: GM54033 (NIGMS)
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1999 Aug 17) 96 (17) 9491-6. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: PDB-1QSS; PDB-1QSY; PDB-1QTM
ENTRY MONTH: 199909
ENTRY DATE: Entered STN: 19990925
Last Updated on STN: 19990925
Entered Medline: 19990909

AB The Taq DNA polymerase is the most commonly used enzyme in DNA sequencing.
However, all versions of **Taq polymerase** are deficient in two respects: (i) these enzymes incorporate each of the four dideoxynucleoside 5' triphosphates (ddNTPs) at widely different rates during sequencing (ddGTP, for example, is incorporated 10 times faster than the other three ddNTPs), and (ii) these enzymes show uneven band-intensity or peak-height patterns in radio-labeled or dye-labeled DNA
sequence profiles, respectively. We have determined the crystal structures
of all four ddNTP-trapped closed ternary complexes of the large fragment of the Taq DNA polymerase (Klentaq1). The ddGTP-trapped complex structure differs from the other three ternary complex structures by a large shift in the position of the side chain of residue 660 in the O helix, resulting
in additional hydrogen bonds being formed between the guanidinium group of
this residue and the base of ddGTP. When Arg-660 is mutated to Asp, Ser, **Phe**, **Tyr**, or Leu, the enzyme has a marked and selective reduction in ddGTP incorporation rate. As a result, the G track generated during DNA sequencing by these **Taq polymerase variants** does not terminate prematurely, and higher molecular-mass G bands are detected. Another property of these **Taq polymerase variants** is that the sequencing patterns produced by these enzymes are remarkably even in band-intensity and peak-height distribution, thus resulting in a significant improvement in the accuracy of DNA sequencing.

L6 ANSWER 2 OF 3 MEDLINE
ACCESSION NUMBER: 1999129939 MEDLINE
DOCUMENT NUMBER: 99129939 PubMed ID: 9931007
TITLE: Functional roles of the conserved aromatic amino acid

residues at position 108 (motif IV) and position 196 (motif VIII) in base flipping and catalysis by the N6-adenine DNA methyltransferase from Thermus aquaticus.

AUTHOR: Pues H; Bleimling N; Holz B; Wolcke J; Weinhold E
CORPORATE SOURCE: Max-Planck-Institut fur molekulare Physiologie, Abteilung Physikalische Biochemie, Dortmund, Germany.
SOURCE: BIOCHEMISTRY, (1999 Feb 2) 38 (5) 1426-34.
Journal code: A0G; 0370623. ISSN: 0006-2960.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199902
ENTRY DATE: Entered STN: 19990311
Last Updated on STN: 19990311
Entered Medline: 19990223

AB The DNA methyltransferase (Mtase) from Thermus aquaticus (M.TaqI) catalyzes the transfer of the activated methyl group of S-adenosyl-L-methionine to the N6 position of adenine within the double-stranded DNA sequence 5'-TCGA-3'. To achieve catalysis M.TaqI flips the target adenine out of the DNA helix. On the basis of the three-dimensional structure of M.TaqI in complex with the cofactor and its structural homology to the C5-cytosine DNA Mtase from Haemophilus haemolyticus, **Tyr** 108 and **Phe** 196 were suggested to interact with the extrahelical adenine. The functional roles of these two aromatic amino acid residues in M.TaqI were investigated by mutational analysis. The obtained **mutant** Mtases were analyzed in an improved kinetic assay, and their ability to flip the target base was studied in a fluorescence-based assay using a duplex oligodeoxynucleotide containing the fluorescent base analogue 2-aminopurine at the target position. While the **mutant** Mtases containing the aromatic amino acid Trp at position 108 or 196 (Y108W and F196W) showed almost wild-type catalytic activity, the **mutant** Mtases with the nonaromatic amino acid Ala (Y108A and F196A) had a strongly reduced catalytic constant. Y108A was still able to flip the target base, whereas F196A was strongly impaired in base flipping. These results indicate that **Phe** 196 is important for stabilizing the extrahelical target adenine and suggest that **Tyr** 108 is involved in placing the extrahelical target base in an optimal position for methyl group transfer. Since both aromatic amino acids belong to the conserved motifs IV and XIII found in N6-adenine and N4-cytosine DNA Mtases as well as in N6-adenine RNA Mtases, a similar function of aromatic amino acid residues within these motifs is expected for the different Mtases.

L6 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER: 1998:703388 CAPLUS
DOCUMENT NUMBER: 129:327733
TITLE: Mutagenesis of Pol-II type DNA polymerases
INVENTOR(S): Mamone, Joseph A.
PATENT ASSIGNEE(S): Amersham Life Science Inc., USA
SOURCE: U.S., 23 pp.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 5827716	A	19981027	US 1996-688649	19960730

AB A Pol-II type DNA polymerase wherein an alanine located at the nucleotide binding site is replaced with a hydroxy contg. amino acid in order to

prep. polymerases with decreased exonuclease activity. The polymerases may be derived fr[redacted] Pyrococcus furiosus, Thermococ[redacted] litoralis, or Sulfolobus solfataricus. Thus, **mutant** forms of P. furiosus DNA polymerase were constructed with Ala-491 replaced by **Tyr**, Asn-492 replaced with **Tyr**, a **Tyr** residue inserted between Ala-491 and Asn-492, and amino acids 489-494 (LLANSF) replaced with 7 corresponding amino acids (TINYGVL) from **Taq polymerase** including an F.fwdarw.Y substitution. All the **mutants** had significantly reduced specific specific activities (>100-fold of wild type).